

Application No. 09/856,339  
Amendment dated November 29, 2005  
Reply to Office Action of June 29, 2005

Docket No.: HO-P02196US0

**c) Remarks**

As of the outstanding office action, claims 2-3, 5, and 22-25 were pending. As of this response, claims 3, 5, and 22-25 are pending. The following amendments are made in this response:

Claim 25 has been amended as follows:

-to specify that the substituent is an alkyl or alkenyl of 1 to 6 carbon atoms based on page 3, lines 27 and 28 of the application,

-to specify that the mutant enzymes have a higher oxidation activity than the equivalent wild type enzyme,

-to delete references to the use of homologous enzymes,

-to replace the term "variant" with "mutant",

-to replace the term "at at" with "of at",

-to introduce commas as required by the Examiner, and

-to delete the phrase "corresponding to".

The definition of the mutation has also been amended in claim 25 to specify that at least one of the defined mutations is a substitution to an amino acid with a less polar side chain. It is apparent from page 6 lines 24 to 30 of the specification that not all of the mutations are required to be substitutions to an amino acid with a less polar side chain.

Claim 5 has been amended to replace the nomenclature used to designate a double mutation. In addition minor amendments have been made to claims 3 and 5 to clarify the fact that the relevant enzyme has "at least" the specified mutations (which allows for the fact that the P450<sub>cam</sub> mutant enzyme of claim 25 has at least two mutations). Claim 2 has been canceled.

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The following objections/rejections are pending as of this office action. The rejections are addressed in turn.

1. Claims 2-3, 5, and 22-25 are objected to because of various informalities.
2. Claims 2-3, 5, and 22-25 are rejected under 35 USC § 112, second paragraph.
3. Claims 2-3, 5, and 22-25 are under 35 USC § 112, first paragraph.
4. Claims 2-3, 5, and 22-25 are rejected under 35 USC § 103(a) as being unpatentable over U.K. Patent GB 2 294 692 A, U.K. Patent Application GB 2 306 485 A or U.S. Patent 6,117,661 to Wong et al. (hereinafter "Wong").
5. Claims 2-3, 5, and 22-25 are rejected under 35 USC § 103(a) as being unpatentable over U.S. Patent 6,100,074 to Flitsch et al. (hereinafter "Flitsch").

Each objection/rejection is addressed in turn.

1. Objection to Claims 2-3, 5, and 22-25.

In response to the Examiner's objections to the claims on page 3 of the office action, applicants have amended claim 25 to introduce the required commas, to replace the term "at at" with "of at" and to replace the term "variant" with "mutant". Because claim 2 has been canceled the objection to this claim is obviated.

2. Rejection of Claims 2-3, and 22-25 under 35 USC § 112, Second Paragraph

The examiner has rejected claims 2-3, and 22-25 under 35 USC § 112, second paragraph, for indefiniteness.

Claim 2 has been canceled. Thus, the pending rejections with respect to claim 2 are no longer relevant.

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In claim 25, the examiner asserts that "a P450<sub>cam</sub> variant enzyme corresponding to SEQ ID NO: 1" and "a P450<sub>BM-3</sub> variant enzyme corresponding to SEQ ID NO: 24" do not have clear metes and bounds.

The claims have been amended to delete the phrase "corresponding to" and to refer directly to specific SEQ ID's as suggested by the examiner. Applicants believe that this overcomes the rejection.

In claim 25, the examiner asserts that the phrases "comprising at least two or more mutations at amino acid positions which are equivalent to..." and "comprising at least one or more mutations at amino acid positions which are equivalent to..." do not have clear metes and bounds. Thus the examiner asserts that this claim and any dependent claims are indefinite as per §112, second paragraph.

The claims have been amended to delete references to homologous mutant enzymes and therefore these objections in the office action based on this language are no longer relevant. The claims have been amended to remove the "equivalent to" language which the examiner finds indefinite. Applicants believe that this overcomes the rejection.

In claims 22-23, and 25, the examiner asserts that the phrase "substituted derivative thereof" does not have clear metes and bounds. Thus the examiner asserts that these claims and any dependent claims are indefinite as per §112, second paragraph.

The substituted derivatives recited in claim 25 have now been limited to substituents which are either an alkyl or alkenyl of 1 to 6 carbons. Given that the substituents have now been defined, claim 25 is now clear in this regard and the applicants believe that this rejection has been overcome.

In claim 25, the examiner asserts that the phrase "homologue variant enzyme" does not have clear metes and bounds. Thus the examiner asserts that this claim and any dependent claims are indefinite as per §112, second paragraph.

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References to homologous variant enzymes have been deleted from the claims and therefore rejections in the office action based on this language are no longer relevant. Applicants believe that this overcomes the rejection.

In claims 3 and 25, the examiner asserts that the phrase "less polar side-chain" does not have clear metes and bounds. Thus the examiner asserts that this claim and any dependent claims are indefinite as per §112, second paragraph. The examiner asserts that the phrase "less polar side-chain" is indefinite. Applicants respectfully traverse this rejection.

Applicants assert that whether a given side-chain is less polar than another side-chain may be unambiguously determined using the disclosure in the application. The polarity of a side-chain will be determined by its hydropathy value. Lower hydropathy values correspond to more polar properties. Thus in order to choose amino acid with a less polar side-chain than a given amino acid, one merely has to choose an amino acid which is above the given amino acid in Table 3 (as mentioned at page 10, lines 7 and 8 of the specification). Applicants believe that this overcomes the rejection and respectfully request the examiner to withdraw this rejection.

In claim 5, the examiner asserts that the phrase "F87A-I395F" and "R47L-Y51F" does not have clear metes and bounds. The examiner asserts that it is not clear if the mutant enzyme comprises a range of mutations starting from the amino acid at position 87 through the amino acid at position 395 or if the mutant enzyme comprises double mutations at amino acid positions 87 and 295 or at amino acid positions 47 and 81.

Claim 5 has been amended to replace the references to double mutations with a written description of the mutations. Thus this claim is now clear.

In light of the amendments and arguments made herein, applicants assert that all of the outstanding rejections under 35 USC § 112, second paragraph have been addressed and that the claims are in condition for allowance. Accordingly, applicants respectfully request that the rejections under 35 USC § 112, second paragraph be withdrawn.

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3. Rejection of Claims 2-3, 5, and 22-25 under 35 USC § 112, First Paragraph

The examiner has rejected claims 2-3, and 22-25 under 35 USC § 112, first paragraph, for lack of enablement in the specification. The examiner asserts that the specification, while being enabling for a method of oxidizing pinene, limonene or cyclic sesquiterpene using a variant of P450<sub>BM-3</sub> with SEQ ID NO: 24 or a variant of P450<sub>cam</sub> with SEQ ID NO: 1 comprising an amino acid sequence that has at least 95 % sequence identity to SEQ ID NO:1 or 24 and consists of the recited amino acid mutations (amino acid 87, 96, 244, 247, or 248 of P450<sub>cam</sub> or amino acids 47, 51, or 87 of P450<sub>BM-3</sub>, it does not reasonably provide enablement for a method of oxidizing pinene, limonene, any cyclic sesquiterpene, or substituted derivatives of the same using any variant of P450<sub>BM-3</sub> of SEQ ID NO: 24 or P450<sub>cam</sub> of SEQ ID NO: 1.

References to homologous enzymes have been deleted from base claim 25 and therefore this objection is no longer relevant. Claim 2 has been canceled. Applicants assert that the amendments to the claims overcome this rejection. Applicants respectfully request that the examiner withdraw this rejection.

4. and 5. Rejection of Claims 2-3, 5, and 22-25 under 35 USC § 103(a) over Wong and  
Rejection of Claims 2-3, 5, and 22-25 under 35 USC § 103(a) over Flitsch

The examiner has rejected claim 2-3, 5, and 22-25 under 35 USC § 103(a) over Wong, and has also rejected the same claims under 35 USC § 103(a) over Flitsch. Applicants respectfully traverse both rejection under § 103(a).

From the examiner's comments it seems that the subject matter relating to P450<sub>BM-3</sub> mutant enzymes is considered to be non-obvious, and therefore the only outstanding issue relates to the subject matter of relating to P450<sub>cam</sub> mutant enzymes. The following arguments are in respect of such subject matter.

The examiner has maintained that the claims are obvious from Wong et al. and Flitsch et al. The examiner's reasoning is essentially based on similarities that are identified between the

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enzymes and substrates mentioned in the present claims and the enzymes and substrates which are mentioned in the cited documents. However for the reasons set out below we believe that the examiner's conclusion is incorrect because the examiner has not appreciated numbers of (and extent of) differences between the method of the present claims and the methods of the cited documents.

Wong and Flitsch disclose results from the testing of a large number of compounds. It is noteworthy that the substrates referred to in the present claims are not disclosed in the cited documents. Instead the cited documents test many related compounds which are either aromatic or alkanes or compounds which have halogen or oxygen substituents (and thus would be chemically activated as a consequence). The skilled artisan, reading the cited documents, would recognize that the compounds which are disclosed fell into these broad groups. The skilled artisan would not be motivated to test any other group of compounds.

The cited documents do not provide any detailed teaching which allows prediction of which substrates would be oxidized by mutant P450<sub>cam</sub> enzymes. The examiner is using her own knowledge to assert that the skilled person would have been motivated based on the cited documents to test whether the substrates mentioned in the claims could be oxidized by mutant. In particular the examiner has referred to the single disclosure of isoprene as evidence that the skilled person would have been motivated to test the substrates mentioned in the claims. However isoprene is a very small molecule in comparison to limonene and pinene, and in particular in comparison to sesquiterpenes. Although the examiner has identified one similarity between isoprene and the substrates mentioned in the claims (i.e. they are all terpenes), the skilled person would not view this as sufficient to predict whether or not the substrates of the claims could be oxidized by the same enzyme.

In the declaration filed with the previous response, inventor Dr. Wong discusses in detail the criteria that would be considered by the skilled artisan when assessing whether the much larger molecules mentioned in the claims would also be oxidized by the same enzyme which oxidizes isoprene. As discussed in paragraphs 6 to 9 of the declaration, a person of ordinary skill

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in the art would need to assess whether an active site which is known to oxidize small molecules or flat planar aromatic molecules would be expected to oxidize large molecules which have a substantial 3D bulk. There is no indication in the cited documents that the active site is able to accommodate such molecules, and further is able to successfully oxidize such molecules.

When judging the motivation for the skilled person to test the substrates mentioned in the claims after reading the cited documents, the examiner must take into account the criteria which the skilled person would consider in predicting whether or not a given compound could be oxidized. Applicants respectfully submit that the examiner has not done this. The skilled person reading the cited documents would only be led to consider compounds within the broad groups mentioned above (i.e., planar aromatic and alkanes and compounds activated by a halogen or oxygen). The skilled person would not focus on the disclosure of isoprene and consider testing much larger molecules in the same chemical group, especially given the knowledge that the active site of an enzyme will impose limitations as to which substances it will successfully oxidize. It is not realistic to propose that the skilled person would simply have proceeded to test the substances mentioned in the claims, and thus the examiner is viewing the invention through hindsight.

Further the present claims refer to other features which are not disclosed or appreciated in the disclosure of the cited documents. In particular the present claims are directed to the use of enzymes with a particular combination of mutations to oxidize particular substrates. Although the cited documents mention that the enzyme may have more than one mutation there is no appreciation of the fact that multiple mutations lead to a higher oxidation activity than single mutations. As the Examiner points out in the last sentence on page 9 of the office action, "one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions". As discussed in the declaration and data filed with the previous response multiple substitutions in the active site of P450<sub>CAM</sub> do in fact lead to increased activity against the substrates mentioned in the claims.

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The examiner's comments, in fact, support the argument that the skilled person would expect mutations in the active site to disrupt (and thus diminish) oxidation activity (by causing disruption to the activity of the amino acids which are involved in the oxidation reaction), and thus provide evidence of the fact that the person of ordinary skill in the art would have no reason to assume that multiple mutations would provide increased activity.

Further as discussed in paragraph 5 of the declaration filed with the previous response, the cited documents contain no appreciation of the surprising advantages that can be obtained by use of multiple mutations. Table 6 on page 47 of the application demonstrates that manipulation of the multiple mutations may be used obtain certain specific products. The cited documents do not teach that the oxidation product which is obtained may be altered by use of specific multiple mutations, and in particular that use of multiple mutations can be used to increase the yield of a single desired product (to 80% or more of the total products formed).

In response to the examiner's specific comment that the claims are not restricted to mutant enzymes with multiple mutations, we wish to draw to the examiner's attention that the claims are restricted to the use of P450<sub>cam</sub> enzymes with at least two specified mutations. The claims cover the use of P450<sub>BM-3</sub> with single mutations, but as mentioned above we assume that the examiner consider such subject matter to be non-obvious.

Further in response to comments that the claims do not recite limitations that the enzymes have a high oxidation activity, the claims have been amended to state that the oxidation activity towards the substrate is higher than the wild-type enzyme.

Thus, in conclusion for the above reasons it is submitted that the claimed method would not be obvious from either Wong or Flitsch. Accordingly, applicants respectfully request that the examiner withdraw the rejection of claims 2-3, 5, and 22-25 under 35 USC § 103(a) over Wong, and the rejection of claims 2-3, 5, and 22-25 under 35 USC § 103(a) over Flitsch.



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**d) Conclusion**

In light of the Applicants' amendments and arguments, applicants assert that the pending claims are in condition for allowance. Applicants respectfully request withdrawal of the outstanding rejections and allowance of the pending claims. If any issues remain outstanding, please contact the undersigned for resolution of the same.

Applicants provide a check in the amount of \$225.00 for a two-month extension of time. Applicants believe that no other fees are associated with the filing of this response. However, if Applicants are in error, the Commissioner is hereby authorized to charge any additional fees associated with this filing from Deposit Account No. 06-2375, under Order No. P02196US0/10104571 from which the undersigned is authorized to draw.

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Respectfully submitted,

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